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by

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SUMMARY

The daily egg production of Pacific sardine (*Sardinops sagax*) off California from San Diego to San Francisco was estimated to be 0.96/0.05m^2 (CV = 0.24). The spawning biomass was estimated to be 281,639.27 metric tons (mt, CV = 0.3) for an area of 320,619.8 km^2, using the daily specific fecundity (number of eggs/population weight (g)/day of 21.86 compared to 23.55 used in the years prior to 2002 and 22.94 from the 2002 cruise). The area (320,620 km^2) is similar to that in 2003 (365,906 km^2). We also revisited sardine egg data collected during the April 1995 CalCOFI survey. The daily egg production for 1995 was 0.83/0.05m^2 (CV = 0.5) for an area of 113,189 km^2. Using the daily specific fecundity from 1986-1994: 23.55 eggs/gram/day, the estimate of the spawning biomass in1995 was 79,997 mt (CV = 0.6). The estimates of spawning biomass of Pacific sardine in 1994 - 2004 are 127,000 mt, 80,000 mt, 83,000 mt, 410,000 mt, 314,000 mt, 282,000 mt, 1.06 million mt, 791,000 mt, 206,000 mt, and 485,000 mt, 300,000 mt respectively. Therefore, the estimates of spawning biomass have been fluctuating and increasing since 1994.

INTRODUCTION

The spawning biomass of Pacific sardine (*Sardinops sagax*) was estimated independently during 1986 (Scannel et al. 1996), 1987 (Wolf 1988a), 1988 (Wolf 1988b), 1994 (Lo et al. 1996), and 1996 (Barnes et al. 1997), using the daily egg production method (DEPM: Lasker 1985). DEPM estimates spawning biomass by: 1) calculating the daily egg production from ichthyoplankton survey data, 2) estimating the maturity and fecundity of females from adult fish samples, and 3) calculating the biomass of spawning adults. Before 1996, sardine egg production was estimated from direct CalVET plankton net sampling. Adult fish were sampled in various ways to obtain specimens for batch fecundity, spawning fraction, sex ratio, and average female fish weight prior to 1996 (Wolf 1988a, 1988b; Scannell et al. 1996; Macewicz et al. 1996; Lo et al. 1996).

Since 1996, in addition to CalVET and Bongo nets, the Continuous Underway Fish Egg Sampler (CUFES; Checkley, et al. 1997) has been used as a routine sampler for fish eggs, and data of sardine eggs with CUFES have been incorporated in various ways depending on the survey design in the estimation procedures of the daily egg production. In the 1997 sardine egg survey (Hill et al. 1998, Lo et al. 2001), CUFES was used to allocate CalVET tows in an adaptive sampling plan. From 1998 to 2000, data of sardine eggs collected with both CalVET and CUFES during each April CalCOFI cruise were used to estimate daily egg production (Hill et al. 1999). Use of the full data sets from both samplers in the DEPM can be time consuming. Furthermore, the CUFES samples are exclusively from 3 m depth and it is not clear whether the distributions of sardine egg stages from CUFES samples are representative. Use of the CUFES data also requires an estimated conversion factor from eggs/min to eggs/0.05m^2. Starting with the 1999 April CalCOFI survey, an adaptive allocation survey design similar to the 1997 survey was implemented. In this design, CalVET tows are added in areas where they were not preassigned if sardine egg densities in CUFES collections are high.

Since 2001, a cost-effective alternative has been adopted to retain the DEPM index, but in a revised form that reduces effort in calculation and egg staging for CUFES collections. This revised DEPM index uses only CalVET samples of eggs and yolk-sac larvae in the high density area (Region 1) to provide an estimate of P_0, the variance of which can be large due to small sample size (normally < 100 plankton tows).

In 2004, a full-scaled survey was conducted for collection of sardine eggs, larvae, and adults to estimate the spawning biomass of Pacific sardine. Adult sardine samples for reproductive output were taken aboard the commercial fishing vessel F/V *Frosti*. The ichthyoplankton samples were taken aboard the R/V *New Horizon* and R/V *David Starr Jordan*.

As we prepared for the Sardine symposium of the 2004 CalCOFI Annual meeting, we revisited sardine egg data collected during 1995 April CalCOFI cruise (April 4 - 22) from 42 stations. To preserve the continuity of this report, we include the estimate of the spawning biomass of 1995 in an appendix.

MATERIALS AND METHODS

Data

Sardine eggs collected with both CalVET and CUFES on the April 2004 *New Horizon* and *Jordan* cruises were the data sources for estimating the daily egg production of sardine. In addition to sardine eggs and yolk-sac larvae collected with the CalVET net, yolk-sac larvae collected with the Bongo net have been included to model the sardine embryonic mortality curve since 2000. As in 2001 (Lo 2001), the CUFES data from the 2004 survey were used only to map the spatial distribution of sardine spawning population. The survey area was later post-stratified into high density and low density areas according to the egg density from CUFES collections. Staged eggs from CalVET tows and yolk-sac larvae from CalVET and Bongo tows in the high density area were used to model embryonic mortality curve in the high density area and later converted to the daily egg production, P_0, for the whole survey area.

During the 2004 survey, the regular CalCOFI survey was extended to CaCOFI line 60.0 (San Francisco) with the *New Horizon* cruise (March 23 - April 9) occupying six regular CalCOFI lines (93.3 - 76.6) and the *Jordan* cruise (April 13 - 25) occupying five northern lines (73.3- 60.0). Therefore, the total number of lines occupied by both vessels was 11 lines with lines 40 nm apart. Bongo samples were taken only at regular CalCOFI survey stations aboard both the R/V *New Horizon* in the south and the R/V *David Starr Jordan* in the north. On the southern 6 lines CalVET tows were taken only at regular CalCOFI survey stations. For the *Jordan* cruise, occupying the northern 5 lines, CalVET tows were taken at 4 nm intervals on each line after the egg density from each of two consecutive CUFES samples exceeded 1 egg/min. Similarly, CalVET tows were stopped after the egg density from each of two consecutive CUFES samples was less than 1 egg/min. The threshold of 1 egg/min was reduced from the number used in years prior to 2002 (2 eggs/min) to increase the area identified as the high density area and, subsequently, to increase the number of CalVET samples. This adaptive allocation sampling was similar to the 1997 survey (Lo et al. 2001).

The survey area was post-stratified into two regions: Region 1, the high density area, and Region 2, the low density area. Region 1 encompassed the area where the egg density (eggs/min) in CUFES collections was at least 1 per minute. The rest of the survey area was Region 2 (Figure 1). One egg/min is equivalent to two to seven eggs/CalVET tow, depending on the degree of water mixing.

A total of 781 CUFES samples was collected from both the *New Horizon* (432) and *Jordan* (349) cruises. CUFES sampling intervals ranged from 1 to 60 minutes with a mean of 26 minutes and median of 30 minutes. A total of 124 CalVET samples was collected, of which 67 contained at least one sardine egg (Table 1). Egg densities from each CalVET sample and from the CUFES samples taken within an hour before and after the CalVET tow, were paired and used to derive a conversion factor (*E*) from eggs/min of CUFES sample to CalVET catch. We used a regression estimator to compute the ratio of mean eggs/min from CUFES to mean eggs/tow from CalVET: $E = \mu_y / \mu_x$ where y is the eggs/min and x is eggs/tow.

Daily egg production (P_0)

Similar to the 2001 procedure (Lo 2001), we used the net tow as the sampling unit. Eggs from CalVET tows and yolk-sac larvae from both CalVET and Bongo tows in Region 1 were used to compute egg production based on data from the 11 transects (lines 60-95) (Figure 1). A total of 63 of the 71 CalVET samples in this region contained ≥1 sardine egg; these eggs were examined for their developmental stages (Figure 2).

Based on aboard-ship count of CUFES samples, among the 781 collections, 251 were positive for sardine eggs. In Region 1, there were 164 positive CUFES collections out of 181 total collections. In Region 2, 87 of the total 600 collections were positive (Table 1).

For purpose of modeling the embryonic mortality curve, yolk-sac larvae (larvae ≤5 mm in captured length) were included assuming the mortality rate of yolk-sac larvae was the same as that of eggs (Lo 1986). Yolk-sac larval production was computed as the number of yolk-sac larvae/0.05m^2 divided by the duration of the yolk-sac stage (number of larvae/0.05m^2/day), and the duration was computed based on the temperature-dependent growth curve (Table 3 of Zweifel and Lasker 1976) for each tow. For yolk-sac larvae caught by the Bongo net, the larval abundance was further adjusted for size-specific extrusion from 0.505 mm mesh (Table 7 of Lo 1983) and for the percent of each sample that was sorted. The adjusted yolk-sac larvae/0.05 m^2 was then computed for each tow and was termed daily larval production/0.05 m^2.

In the entire survey area, 50 of 124 CalVET and 31 of 86 Bongo samples had at least one yolk-sac larva (Figure 3). In Region 1, 46 of 71 CalVET, and 9 of 11 Bongo samples were positive for yolk-sac larvae. In Region 2, 4 of 53 CalVET and 22 of 75 Bongo samples were positive for yolk-sac larvae (Table 1).

Daily egg production in Region 1 ($P_{0,1}$)

Sardine eggs and yolk-sac larvae and their ages were used to construct an embryonic mortality curve (Lo et al. 1996). Sardine egg density for each developmental stage was computed based on CalVET samples (Figure 2). The density of eggs in 2004 was lower than that in 2003 and similar to 2002 (Lo and Macewicz 2002, Lo 2003). The density of stages 6 was highest among all stages. A temperature-dependent stage-to-age model (Lo et. al. 1996) was used to assign age to each stage. Sardine eggs and estimated ages were used directly in nonlinear regression. The 3-h old eggs and eggs older than 2.5 day old were excluded because of possible bias. The average temperature for CalVET tows with ≥1 egg was 13.4˚C, lower than 13.8˚C in 2003, and close to the 13.6˚C average in 2002.

The sardine embryonic mortality curve was modeled by an exponential decay curve (Lo et al. 1996):

$$P_t = P_0 e^{-zt} \qquad [1]$$

where P_t is either eggs/0.05m^2/day from CalVET tows or yolk-sac-larvae/0.05m^2/day from CalVET and Bongo tows, and t is the age (days) of eggs or yolk-sac larvae from each tow. A weighted nonlinear regression was used to estimate two parameters in equation (1) where the weights were 1/SD. The standard deviation (SD) of eggs was 5.78, 7.36 and 12.33, for day one, day two and day three age groups respectively. The high SD for day three age group was due to a high catch of 200 eggs with 101 eggs of age 2.5 day at sea temperature of 13.1˚C. The SD of yolk-sac larval production from CalVETs was 1.006 and the SD of yolk-sac larval production from Bongo samples was 0.85. All the standard deviations were higher than those in 2002 and 2003 (Lo and Macewicz 2002; Lo 2003).

A simulation study (Lo 2001) indicated that $P_{0,1}$ computed from a weighted nonlinear regression based on the original data points has a relative bias (RB) of -0.04 of the estimate where the RB = (mean of 1,000 estimates - true value)/mean of 1,000 estimates. Therefore the bias-corrected estimate of $P_{0,1,c} = P_{0,1} * (1- \text{RB}) = P_{0,1} *(1.04)$, and SE ($P_{0,1,c}$) = SE($P_{0,1}$) * 1.04.

Daily egg production in Region 2 ($P_{0,2}$)

Although 53 CalVET samples were taken in Region 2, only 4 tows had ≥1 sardine egg, ranging from 1 to 28 eggs per tow (Table 1). Therefore, we estimated daily egg production ($P_{0,2}$) as the product of the egg production in Region 1 ($P_{0,1,c}$) and the ratio of egg density in Region 2 to Region 1 (***q***) from CUFES samples, assuming the catch ratio of eggs/min from CUFES to eggs/tow from CalVET is the same for the whole survey area:

$$P_{\mathbf{0,2}} = P_{\mathbf{0,1,}c}q \qquad [2]$$

$$q = \frac{\sum_i \frac{\bar{x}_{2,i}}{\bar{x}_{1,i}} m_i}{\sum_i m_i} \qquad [3]$$

$$\mathbf{var}(q) = \frac{[n/(n-1)]\sum_i m_i^2 (q_i - q)^2}{\left(\sum_i m_i\right)^2}$$

where ***q*** is the ratio of eggs/min between the low density and high density areas, m_i was the total CUFES time (minutes) in the ith transect, $\bar{x}_{j,i}$ is eggs/min of the ith transect in the jth Region, and $q_i = \frac{\bar{x}_{2,i}}{\bar{x}_{1,i}}$ is the catch ratio in the ith transect.

Daily egg production for the whole survey area (P_0)

P_0 was computed as the weighted average of $P_{0,1}$ and $P_{0,2}$:

$$P_0 = \frac{P_{0,1,c}A_1 + P_{0,2}A_2}{A_z + A_2} \qquad [4]$$
$$= P_{0,1,c}w_1 + P_{0,2}w_2$$
$$= P_{0,1,c}[w_1 + qw_2]$$

and

$$mse(P_0)$$
$$= mse(P_{0,1,c})(w_1 + w_2q)^2 + P_{0,1,c}^2 w_2^2 V(q) - mse(P_{0,1,c}) w_2^2 V(q)$$

(Goodman 1960) where $mse\ (P_{0,1,c}) = v(P_{0,1}) + (P_{0,1}\ RB)^2$

and $w_i = \frac{A_i}{A_1 + A_2}$, and A_i is the area size for i - 1, 2.

Adult parameters

The trawl survey was conducted aboard the F/V *Frosti* from April 22 to April 28 after the April CalCOFI ichthyoplankton survey (Figure 4). Adult Pacific sardines were dispersed or in small schools. We conducted trawls near the surface (0-6 fathoms depth) in potential adult sardine areas as identified by the presence of >1 sardine egg per minute in CUFES collection onboard the R/V *Jordan* and the R/V *New Horizon* from March 23 to April 25 and when acoustic signals identified fish schools. A total of 25 trawls was taken of which 17 were positive for sardines.

Up to 50 sardines were randomly sampled from each positive trawl (Table 2). If necessary, additional mature females were collected to obtain 25 mature females per trawl for reproductive parameters or for use in estimating batch fecundity. Each fish was sexed; standard length (mm) and weight (g) were measured; otoliths were removed for aging; and ovaries were removed and preserved in 10% neutral buffered formalin. Each preserved ovary was blotted and weighed to the nearest milligram in the laboratory. Ovary wet weight was calculated as preserved ovary weight times 0.78 (unpublished data, CDFG 1986). A piece of each ovary was removed and prepared as hematoxylin and eosin (H&E) histological slides. All slides were analyzed for oocyte development, atresia, and postovulatory follicle age to assign female maturity and reproductive state (Macewicz et al. 1996).

Four adult parameters are needed for estimation of spawning biomass: 1) daily spawning fraction or the number of spawning females per mature female per day (S); 2) the average batch fecundity (F); 3) the proportion of mature female fish by weight (sex ratio, R); and 4) the average weight of mature females (g, W_f). Population values were estimated by methods in Picquelle and Stauffer (1985). Daily specific fecundity (number of eggs per population weight (g) per day) is RSF/W_f. Correlations among all pairs of adult parameters were calculated for computing the variance of the estimate of spawning biomass (Parker 1985). An MS ACCESS[1] Visual Basic program (Chen et al. 2003) was used to summarize the trawl adult parameters, calculate adult parameter correlations, and to estimate spawning biomass and covariance.

Spawning fraction (S). A total of 290 mature female sardines was analyzed and considered to be a random sample of the population in the area trawled. Histological criteria can be used to identify four different spawning nights: postovulatory follicles aged 44-54 hours old indicated spawning two nights before capture (A); postovulatory follicles aged about 20-30 hours old indicated spawning the night before capture (B); hydrated oocytes or new (without deterioration) postovulatory follicles indicated spawning the night of capture (C); and early stages of migratory-nucleus oocytes indicated spawning the night after capture (D). The daily spawning fraction can be estimated by using the number of females spawning on one night, an average of several nights, or all nights. We used the number of females identified as having spawned the night before capture (B) and the adjusted number of mature females caught in each trawl (Table 2) to estimate the population spawning fraction and variance which is the default spawning night in the EPM program (Chen et al. 2003) and the traditional method of Picquelle and Stauffer (1985).

Batch fecundity (F). Batch fecundity (number of oocytes per spawn) was considered to be the number of migratory-nucleus-stage oocytes or the number of hydrated oocytes in the ovary (Hunter et al., 1985). We used the gravimetric method (Macewicz et al. 1996; Hunter et al. 1985, 1992) to estimate mean batch fecundity for 39 females caught during the April 2004 survey. The relationship of batch fecundity (F_b) to female weight (without ovary, W_{of}), as determined by simple linear regression, was F_b = -2106 + 369.53W_{of} where r^2 = 0.6 but the intercept did not differ from zero (P = 0.790). Therefore, we forced the regression through 0 yielding the relationship F_b = 356.46W_{of} where W_{of} ranged from 70 to 197g (Figure 5). We used this equation to predict batch fecundity for each of the 290 mature Pacific sardine females analyzed to estimate spawning frequency.

Female weight (W_f). The observed female weight was adjusted downward for females with hydrated ovaries because their ovary weights were temporarily inflated. We obtained the adjusted female weight by the linear equation W_f = -4.24 + 1.09W_{of} where W_f is wet weight and W_{of} is ovary-free wet weight based on data from non-hydrated females taken during the April 2004 survey.

Sex ratio (R). The female proportion by weight was determined for each trawl (or each collection). The average weight of males and females (calculated from the first 10 males and 25 females) was multiplied by the number of males or females in the collection of 50 randomly selected fish to calculate total weight by sex in each collection. Thus, the female proportion by weight in each collection (Table 2) was calculated as estimated total female weight divided by estimated total weight in the sample. The estimate of the population's sex ratio by weight was calculated (Picquelle and Stauffer, 1985).

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[1] Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

Spawning biomass (B_s)

The spawning biomass was computed according to:

$$B_s = \frac{P_0 AC}{RSF / W_f} \qquad [5]$$

where A is the survey area in unit of 0.05 m^2, S is the number of females spawning per mature females per day, F is the batch fecundity (number of eggs per mature female), R is the fraction of mature female fish by weight (sex ratio), W_f is the average weight of mature females (g), and C is the conversion factor from grams (g) to metric tons (mt). P_0A is the total daily egg production in the survey area, and the denominator (RSF/W_f) is the daily specific fecundity (number of eggs/population weight (g)/day).

The variance of the spawning biomass estimate $\left(\hat{B}_s\right)$ was computed from the Taylor expansion and in terms of the coefficient of variation (CV) for each parameter estimate and covariance for adult parameter estimates (Parker 1985):

$$VAR\left(\hat{B}_s\right) = \hat{B}_s^{\,2}\left[CV\left(\hat{P}_0\right)^2 + CV\left(\hat{W}_f\right)^2 + CV\left(\hat{S}\right)^2 + CV\left(\hat{R}\right)^2 + CV\left(\hat{F}\right)^2 + 2COVS\right] \qquad [6]$$

The covariance term on the right-hand side is

$$COVS = \sum_i \sum_{i<j} sign \frac{COV\left(x_i, x_j\right)}{x_i x_j}$$

where x's are the adult parameter estimates, and subscripts i and j represent different adult parameters; e.g., $x_i = F$ and $x_j = W_f$. The sign of any two terms is positive if they are both in the numerator of B_S or denominator of B_S (equation 5); otherwise, the sign is negative.

RESULTS

Daily egg production (P_0)

In Region 1, the daily egg production ($P_{0,1}$) (equation 1) was 3.78/0.05 m^2/day (CV=0.23) compared to 2003 data of 5.82/0.05 m^2/day (CV=0.18), egg mortality was Z=0.25 (CV=0.04) compared to 2003 data of 0.48(CV=0.08), and the area was 68,203 km^2 (19,928 nm^2) compared to 2003 data of 82,578 km^2 (24,128 nm^2) (equation 1 and Figure 6). The bias-corrected egg production, ($P_{0,1,c}$) is 3.92 (CV=0.23) (Table 3). The ratio ($\boldsymbol{q}$) of egg density between Region 2 and Region 1 from CUFES samples was 0.042 (CV=0.35) (equation 3). In Region 2, the egg production ($P_{0,2}$) was 0.16 /0.05 m^2/day (CV=0.43) for an area of 252,417 km^2 (73,752 nm^2). The estimate of the daily egg production for the entire survey area was 0.96/0.05 m^2 (CV=0.24) (equation 4) for a total area of 320,620 km^2 (93,680 nm^2) (Table 3). Egg mortality, Z, was much lower than in 2003 (0.48, CV=0.08). Estimates of Z among other years are: 0.4 (CV=0.15) in

2002, 0.37 (CV=0.22) in 2001, 0.42 (CV=0.73) in 2000, 0.1 (CV=0.6) in 1999, and 0.255 (CV=0.37) in 1998 (Table 4).

Catch ratio between CUFES and CalVET (E)

Although this ratio is no longer needed in the current estimation procedure, we computed it for comparison purposes. The catch ratio of eggs/min to eggs/tows (eggs/min = E * eggs/0.05 m^2) was computed from 66 pairs of eggs/0.05 m^2 from CalVET tows and eggs/min from CUFES collections, excluding the maximum tow of 200 eggs as the maximum of eggs in all other tows was fewer than 50 (Figure 7). The eggs/min corresponding to each positive CalVET tow was the mean eggs/min from all CUFES collections taken from one hour before to one hour after each positive CalVET tow. The catch ratio was 0.22 (CV=0.09) compared to 2003 estimate of 0.39 (CV=0.11). A ratio of 0.22 means that one egg/tow from CalVET tow was equivalent to approximately 0.22 egg/min from a CUFES sample, or one egg/minute from the CUFES was equivalent to 4.54 eggs/tow from the CalVET sample.

Adult parameters

Standard length of the *Sardinops sagax* in the samples ranged from 171 to 271 mm for 230 males and from 142 to 278 mm for 344 females. Reproductive parameters of the mature female sardines for the individual trawls are given in Table 2. The population sex ratio (R), based on adult samples from the April 2004 survey, was 0.618 (CV = 0.06) and indicated a possible bias (see discussion); hence, we set R to equal 0.50 in the estimation of spawning biomass (Figure 8). Estimates of the other female sardine parameters were (EPM program of Chen et al. 2003): F, mean batch fecundity, was 55,710.71 eggs/batch (CV = 0.04); S, spawning fraction, was 0.131 per day (CV = 0.17); and W_f, mean female fish weight, was 166.99 grams (CV = 0.02). The average interval between spawning (or spawning frequency) was about 8 days (inverse of spawning fraction or 1/0.131), and the daily specific fecundity was 21.86 eggs/gm/day (Figure 8). The correlation matrix for the adult parameter estimates is shown in Table 4.

Spawning biomass (B_s)

The final estimate of spawning biomass of sardine in 2004 (equation 5) was 281,639 mt (CV=0.36), 309,802 short tons (st) (=281,639 x 1.1) for an area of 320,620 km^2 (93,680 nm^2) from San Diego to San Francisco. The point estimates of spawning biomass of Pacific sardine in 1994-2003 are respectively 127,102; 79,997; 83,176; 409,579; 313,986; 282,248; 1,063,837; 790,925; 206,333; and 485,121 mt (Table 5).

DISCUSSION

Sardine eggs

The distribution of sardine eggs was very different from the past because few eggs were observed south of Point Conception (Figure 1). The egg density was lower than 2003 and similar to that of 2002. The causes of the unusual spatial distribution of the sardine eggs could be that the peak spawning time might have been earlier than April, or some of the spawning population may have moved to the north, or both.

The adaptive allocation sampling procedure was only used aboard R/V *Jordan*, which covered the area north of Point Conception, and not aboard R/V *New Horizon* because the latter was conducting the routine CalCOFI survey. As it turned out, few eggs were observed in the area from San Diego to Point Conception, and it would not have been necessary to allocate more CalCOFI tows in this area. However, if sardine had spawned heavily in the area, we would have missed the opportunity to collect sardine eggs and adult samples and the survey results would not have been satisfactory. We highly recommend that the adaptive allocation sampling be applied aboard the research vessel that conducts the routine CalCOFI survey in April for estimating the spawning biomass of Pacific sardine.

Catch ratio between CUFES and CalVET (E)

The 2004 catch ratio between CUFES and CalVET (0.22) was similar to those obtained in the recent years: 1998 (0.32), 1999 (0.34), 2000 (0.277), and 2001 (0.145(CV=0.026)) 2002 (0.24(CV=0.06)), 2003 (0.39(CV=0.11)). This value of 0.22 was again quite different from the 1996 estimate of 0.73. This could be because the 1996 CalVET samples were taken only in the southern area near San Diego while after 1997 CalVET samples were taken in a larger area much north of San Diego.

Adult parameters

Estimates of all adult reproductive output parameters were obtained from 16 of the 17 positive trawls because one collection contained only a single male. If we used the sex ratio (61.8% females) calculated directly from our 2004 data, the estimated spawning biomass would be 227,746 mt. We set the sex ratio to 50% females in the final estimation of spawning biomass because we felt that a bias may exist as a result of one or more of the following: a) trawling in the region of high egg density only; b) change in depth of trawls from across the upper water column to only surface; c) behavior of the fish; and/or d) large sardines are predominately females because they live longer. We recommend allocating some trawl samples in areas of low egg density to reduce one possible bias. During the 2004 survey, the fraction of mature females spawning on the night of capture (*C*, "Day 0" = 0.131; Figure 8) was the same as the fraction of mature females that spawned the night before capture (*B*, "Day 1" = 0.131). Macewicz et al. (1996) indicated that an average of the spawning fractions from both nights may improve the estimate of *S*. In 2004, we calculated *S* using the number of females spawning during the night before capture only; our estimate may have a slightly higher variance but any bias should be minimum. We recommend continuing the evaluation of the estimates of *S* using females spawning on the different nights to improve the estimate of the fraction of females spawning.

Spawning biomass

The estimate of spawning biomass is considerably lower than that in 2003 but similar to 2002. These differences are primarily due to the change of the egg production, 0.96 eggs/0.05m^2, compared to 1.52 eggs/0.05m^2 in 2003 and 0.728 eggs/0.05m^2 in 2002, while the area of Region 1 was 68,204 km^2, smaller than 83,578 km^2 in 2003 and 88,403 km^2 in 2002 (Table 5). The daily specific fecundity of 21.86 eggs/g/day used for the 2004 estimate of spawning biomass was based on trawl samples taken from Region 1, the high density area. Yet, the daily egg production was an average for the entire survey area. Thus, the spawning biomass may have been underestimated. The degree of underestimation may be minimal unless the daily specific fecundity is substantially lower in Region 2 than that in Region 1 as the total number of eggs produced per day in Region 1 was 87% of total number of eggs produced per day. In order to test the degree of bias of the spawning biomass using the trawl samples from Region 1 only, it is necessary to collect adult samples in the low density area in future years.

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Table 1. Number of positive tows of sardine eggs from CalVET, yolk-sac larvae from CalVET and Bongo, and eggs from CUFES in Region 1 (eggs/min ≥1) and Region 2 (eggs/min <1) for both *New Horizon* (NH) and *Jordan* (Jord) cruises 0404.

		Region 1			Region 2			Total	NH	Jord
		Total	**NH**	**Jord**	**Total**	**NH**	**Jord**			
CalVET eggs	positive	63	0	63	4	0	4	67	0	67
	Total	71	1	70	53	40	13	124	41	83
CalVET yolk-sac	positive	46	0	46	4	1?	3	50	1	49
	Total	71	1	70	53	40	13	124	41	83
Bongo yolk-sac	positive	9	0	9	22	15	7	31	15	16
	Total	11	1	10	75	60	15	86	61	25
CUFES eggs	positive	164	10	154	87	29	58	251	39	212
	Total	181	10	171	600	422	178	781	432	349

Table 2. Individual trawl information, sex ratio[a], and parameters for mature female *Sardinops sagax*, used in estimation of April 2004 spawning biomass.

COLLECTION INFORMATION								MATURE FEMALES						
			Location									Fraction spawning		
Trawl No.	Day	Time	Latitude N	Longitude W	Surface Temp. °C	Number of fish sampled	Proportion of females	Number analyzed	Body weight (g) Ave.	Weight without ovary (g) Ave.	Batch Fecundity Ave.	Night of capture	Night before capture	Number Females Adjusted[b]
1	22	0109	37.270°	124.328°	13.3	50	0.565	25	178.72	167.80	59815	0.080	0.115	26
22	27	2049	37.240°	124.438°	13.5	1	0.000	0	–	–	–	–	–	0
23	27	2359	37.097°	124.612°	14.0	10	0.621	6	178.05	167.65	59761	0.667	0.333	3
8	23	2248	36.688°	123.752°	13.4	3	0.741	2	180.71	169.11	60282	0.500	0.000	1
3	22	2221	36.683°	122.575°	12.3	1	1.000	1	228.00	216.13	77041	0.000	0.000	1
5	23	0414	36.680°	123.219°	13.1	50	0.611	25	176.48	165.97	59160	0.080	0.042	24
9	24	0025	36.674°	123.855°	13.6	3	1.000	3	201.67	189.17	67430	0.000	0.400	5
18	26	2049	36.082°	123.599°	13.8	50	0.827	25	148.88	140.19	49972	0.000	0.167	30
19	26	2253	36.073°	123.589°	13.8	50	0.536	25	155.60	147.10	52435	0.000	0.167	30
20	27	0124	35.934°	123.757°	13.6	50	0.813	25	170.44	160.18	57098	0.200	0.286	28
21	27	0353	35.896°	123.965°	13.6	50	0.351	25	144.72	137.02	48841	0.600	0.231	13
10	24	2046	35.608°	122.920°	13.7	7	0.745	5	185.60	175.29	62484	0.000	0.000	5
11	24	2250	35.526°	123.046°	13.8	49	0.560	25	174.20	161.70	57641	0.000	0.107	28
12	25	0130	35.438°	123.131°	13.8	50	0.512	23	151.78	141.54	50453	0.043	0.043	23
13	25	0330	35.384°	123.140°	13.7	50	0.725	25	173.04	161.22	57469	0.080	0.080	25
15	25	2311	34.927°	122.734°	14.1	50	0.618	25	176.56	163.08	58133	0.120	0.083	24
16	26	0140	34.782°	122.902°	13.9	50	0.599	25	171.20	158.30	56429	0.120	0.083	24

[a]Sex ratio, proportion of females by weight, based on average weights (Picquelle and Stauffer 1985).
[b] Required if the number of females spawning on the night of capture is an overestimate (equation 9 of Picquelle and Stauffer 1985).

Table 3: Egg production (P_0) of Pacific sardine in 2004 based on egg data from CalVET and yolk-sac larval data from CalVET and bongo in Region 1 (eggs/min ≥1) and Region 2 (eggs/min <1), other parameters, and the spawning bongo from *New Horizon* (March 23-April 9) and *Jordan* (April 13-25) cruises.

Parameter	**Region 1**	**Region 2**		**Whole area**	
number of pump samples (excluding the home-bound track)	181	600	781	781	781
n: CalVET	71	53	124	124	124
P_0 / 0.05m^2	*3.92	0.16	0.96	0.96	0.96
CV	0.23	0.43	0.24	0.24	0.24
Area (km^2)	68204	252416	320620	320620	320620
%	21.27	78.73	100	100	100
year for adult samples			1994	2002	2004
Female fish wt (W_f)			82.5	159.25	166.99
Batch fecundity (F)			24283	54403	55711
Spawning fraction (S)			**0.149	0.1739	0.131
Sex ratio (R)			0.537	0.386	0.5
Eggs/g biomass/day (RSF/W_f)			23.55	22.931	21.852
Spawning biomass (mt)			261356	268448	281709
CV				0.36	0.29
Daily mortality (Z)	0.25				
CV	0.04				
eggs/min	3.22	0.12	0.78		
CV	0.11	0.35	0.11		
q = eggs/min in Region 2/eggs/min in Region 1			0.042		
CV			0.35		
E = eggs.min/eggs/tow			0.22		
CV			0.09		
n: bongo	11	75	86		
Area in nm^2	19928	73752	93680	106912	
Spawning biomass (st)			287492	295293	309880

P_0/0.05m^2 was from CalVET only for 2001 and beyond
*3.92 was corrected for bias of P_0.
**average spawning fraction of active females collected 1986-1994 (Table 8 Macewicz et al. 1996)
22.93 was computed from 23 females of 6 hauls in 2002.
The estimate of spawning biomass in this table may be different from Table 4 due to rounding error.

Table 4. The 2004 output from “frmBIOMASS” form in the ACCESS program EPM after input of 2004 parameters, estimation of adult parameters**,** and button selected to ‘Estimate Correlation and Biomass also’ (Figure 8) (Appendix II-24 Chen et al. 2003).

EPM.PRG PROGRAM

Correlation, Biomass and CV

EGG PRODUCTION VALUES

Po (eggs/day - .05 m2):	0.96
CV of Po:	0.23980967569
Area (square kilometers):	320619.8

BIOMASS ESTIMATE

Biomass (m - tons) =	281639.277162
CV (biomass) =	0.29605330157

CORRELATIONS

Parameter	W	F	S	R
Whole - Body Weight (W)		0.53813888	-0.2636919	-0.0198075
Batch Fecundity (F)			-0.1190296	-0.0159456
Fraction Spawning (S)				-0.0642211
Sex Ratio (R)				

Table 5. Estimates of daily egg production (P_0) for the survey area, daily instantaneous mortality rates (Z) from high density area (region 1), daily specific fecundity (RSF/W), spawning biomass of Pacific sardine and average sea surface temperature for the years 1994 to 2004.

Year	P_0 (CV)	Z (CV)	Area (km^2) (Region 1)	$\frac{RSF}{W}$	Spawning biomass (mt)[b](CV)	Ave. Temp. for positive egg or yolk-sac samples (°C)	Mean temperature (°C)	Methods for P_0
1994	0.193 (0.21)	0.120 (0.91)	380,175 (174,880)	11.38	127,102 (0.32)	14.3	14.7	Weighted nonlinear on grouped data
1995	0.830 (0.5)	0.400 (0.4)	113,188.9 (113,188.9)	23.55[a]	79,997 (0.6)	15.5	14.7	Weighted nonlinear on original data
1996	0.415 (0.42)	0.105 (4.15)	235,960 (112,322)	23.55	83,176 (0.48)	14.5	15.0	Composite estimate
1997	2.770 (0.21)	0.350 (0.14)	174,096 (66,841)	23.55	409,579 (0.31)	13.7	13.9	Weighted nonlinear on grouped data
1998	2.279 (0.34)	0.255 (0.37)	162,253 (162,253)	23.55	313,986 (0.41)	14.38	14.6	Composite estimate
1999	1.092 (0.35)	0.100 (0.6)	304,191 (130,890)	23.55	282,248 (0.42)	12.5	12.6	Composite estimate
2000	4.235 (0.4)	0.420 (0.73)	295,759 (57,525)	23.55	1,063,837 (0.67)	14.1	14.4	Composite estimate
2001	2.898 (0.39)	0.370 (0.21)	321,386 (70,148)	23.55	790,925 (0.45)	13.3	13.2	Weighted nonlinear on original data
2002	0.728 (0.17)	0.400 (0.15)	325,082 (88,403)	22.94	206,333 (0.35)	13.6	13.6	Weighted nonlinear on original data
2003	1.520 (0.18)	0.480 (0.08)	365,906 (82,578)	22.94	485,121 (0.36)	13.7	13.8	Weighted nonlinear on original data
2004	0.960 (.24)	0.250 (0.04)	320,620 (68,234)	21.86	281,639 (.3)	13.4	13.7	Weighted nonlinear on original data

[a] from Table 3: computation for 1994 after setting *S* to 0.149 (the average spawning fraction of active females from 1986-1994; Macewicz et al. 1996)

[b] $cv(B_s)=(cv^2(P_0)+\text{allother }COV^2)^{1/2}=(CV^2(P_0)+0.054)^{1/2}$. For years 1995-2001 allotherCOV^2 was from 1994 data.(Lo et al. 1996). For year 2003, allotherCOV was from 2002 data (Lo and Macewicz 2002)

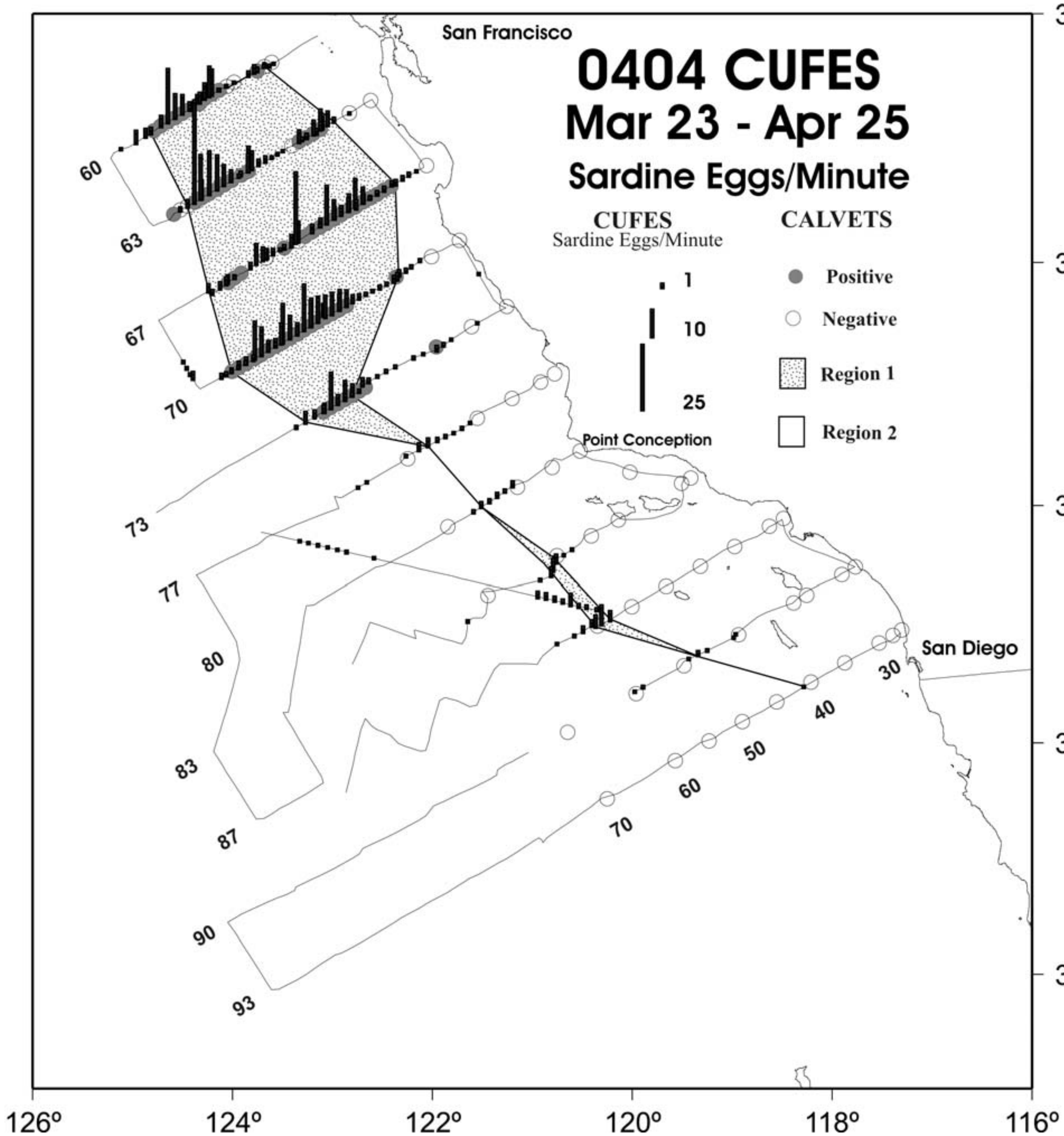

Figure 1. Sardine eggs from CalVET (or Pairovet; solid circle denotes positive catch and open circle denotes zero catch) and from CUFES (stick denotes positive collection) in March-April 2004 survey. The numbers on line 93 are CalCOFI station numbers. Region 1 is stippled area.

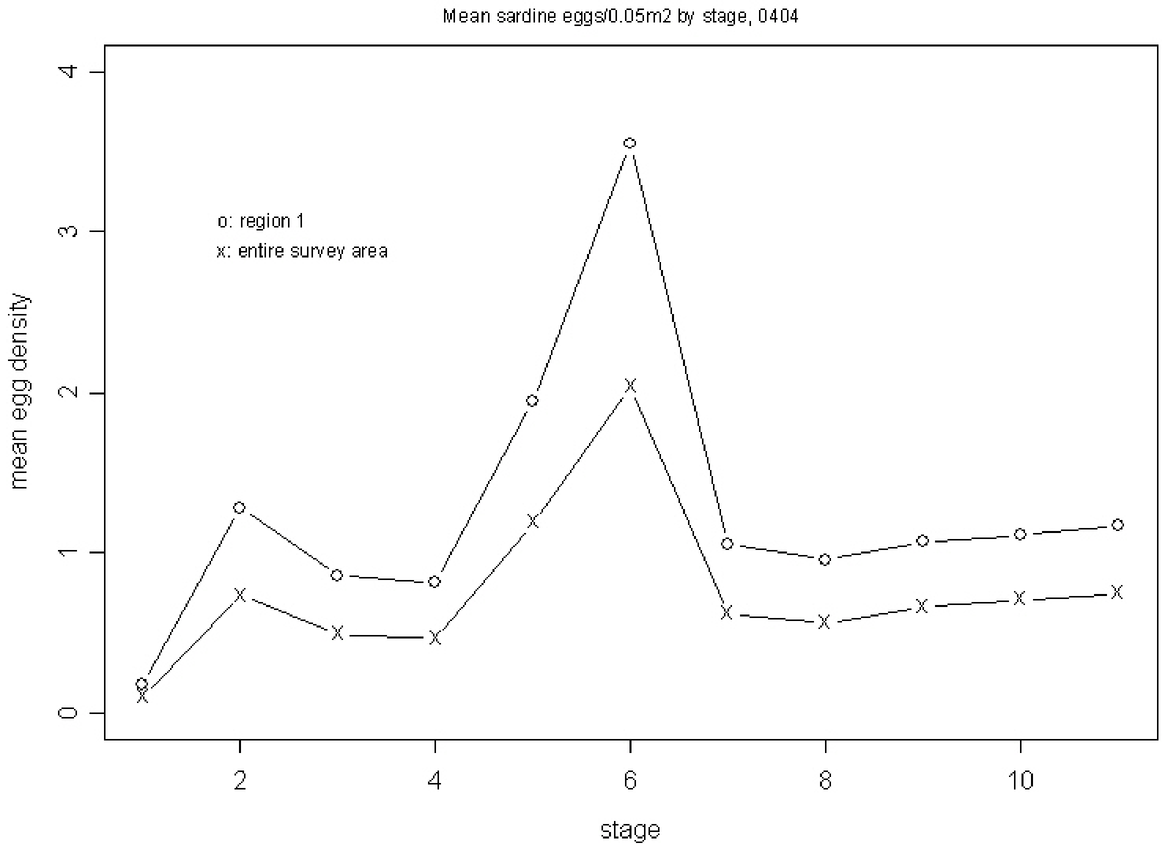

Figure 2. Sardine eggs per $0.05m^2$ for each developmental stage for March-April, 2004.

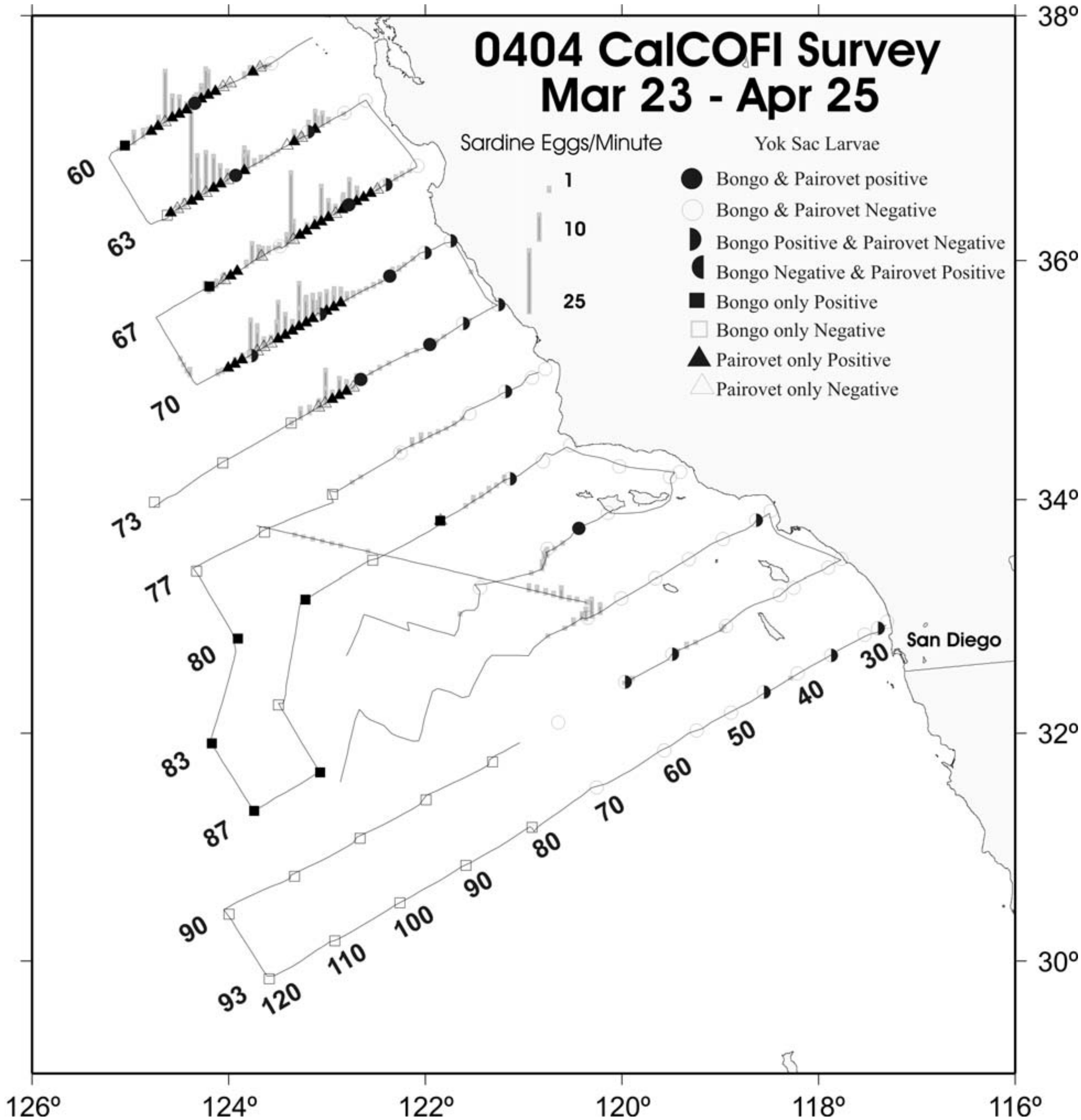

Figure 3. Sardine yolk-sac larvae from CalVET (or Pairovet; circle and triangle) and from Bongo (circle and square) in March-April 2004 survey. Solid symbols are positive and open symbols are zero catch.

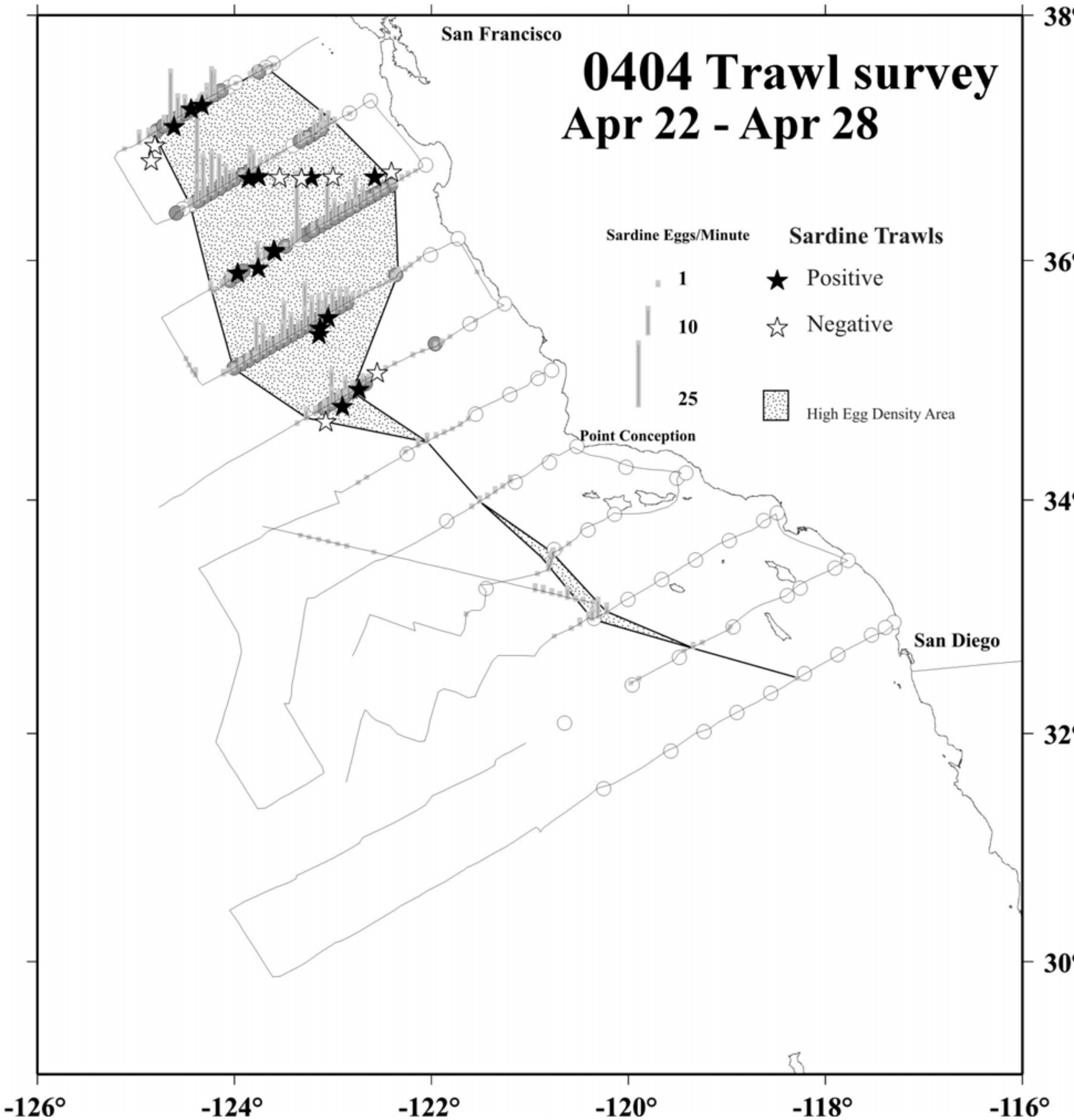

Figure 4. April 2004 trawl start locations. Solid stars are trawls that contained at least one sardine. Background is CUFES track lines and sardine egg abundance, pairovet tows (circles: solid are positive for sardine eggs or larvae; open are negative). The high egg density area is Region 1.

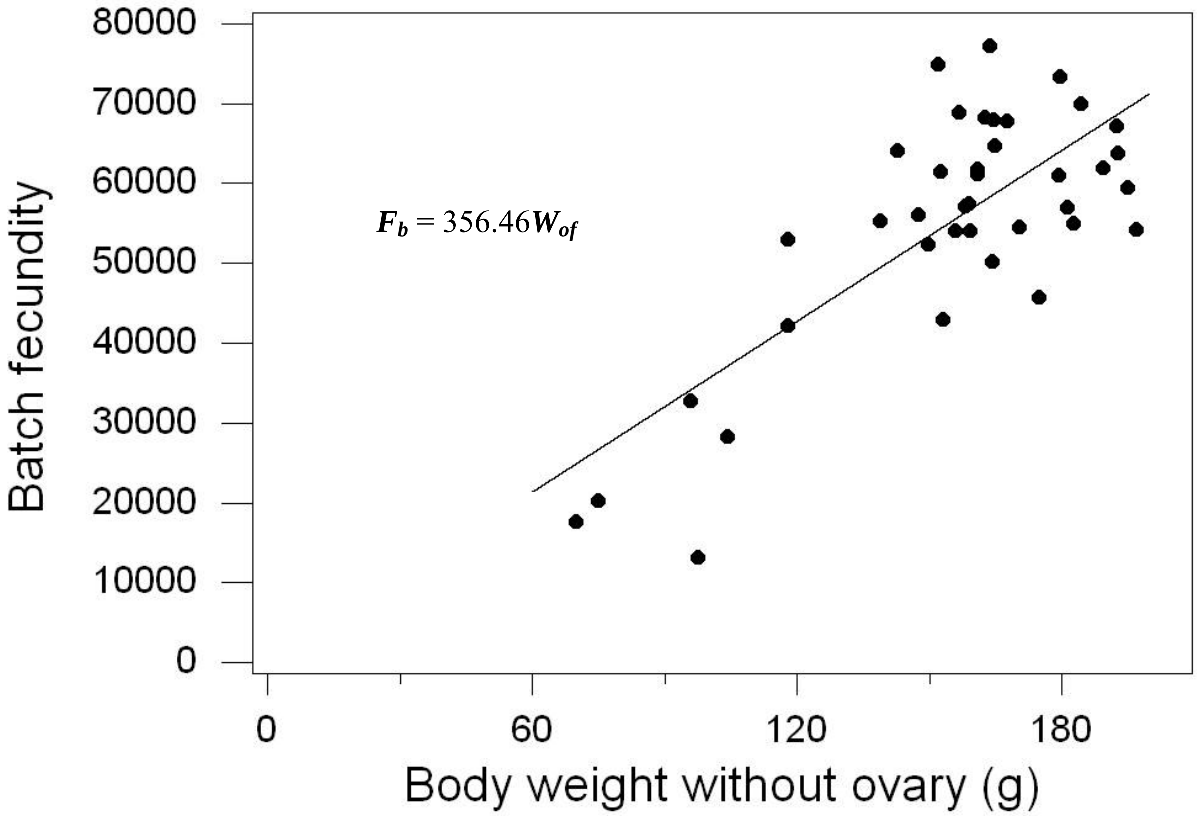

Figure 5. Batch fecundity (F_b) of *Sardinops sagax*as a function of female body weight (W_{of}, without the ovary) for 39 females taken during April 2004. The fitted regression line was $F_b = 356.46W_{of}$. The batch was estimated from numbers of hydrated or migratory-nucleus-stage oocytes.

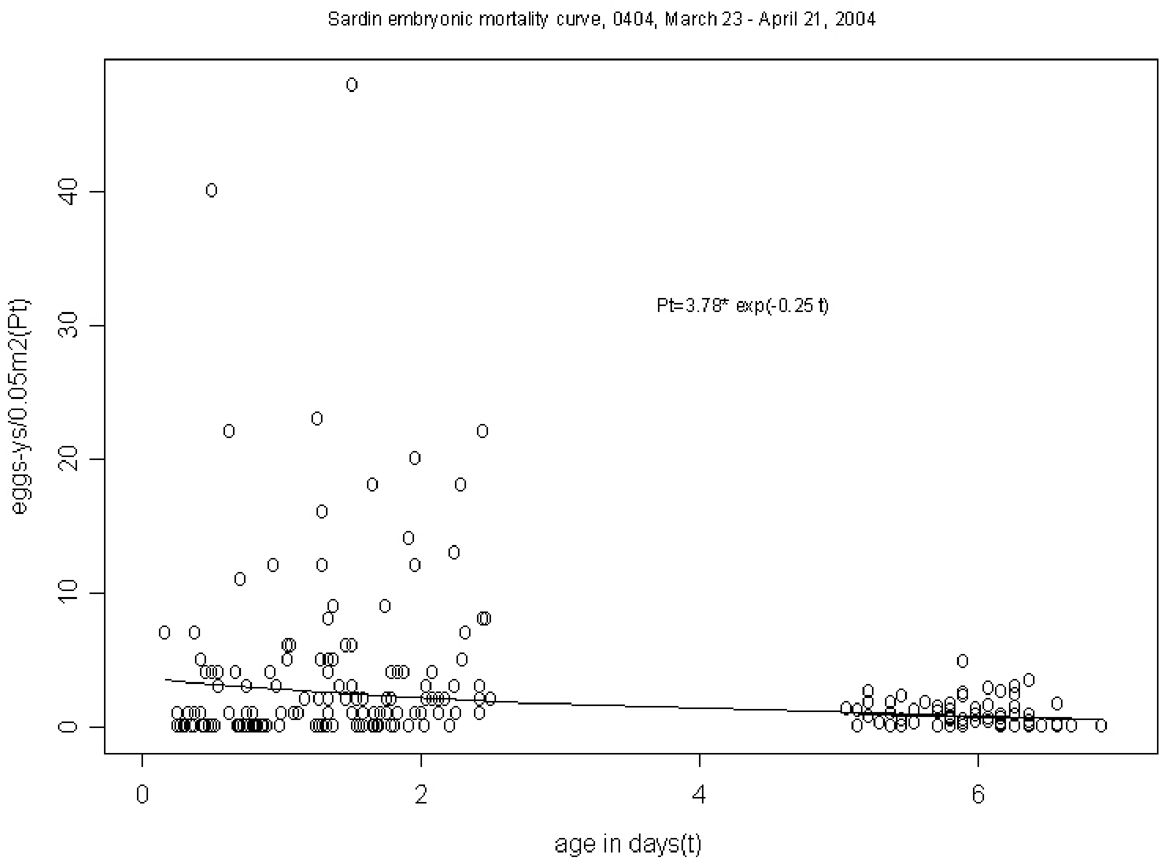

Figure 6. Embryonic mortality curve of Pacific sardine. Staged egg data were from CalVET and yolk-sac larval data were from CalVET and Bongo. The number, 3.78, is the estimate of daily egg production before correction for bias.

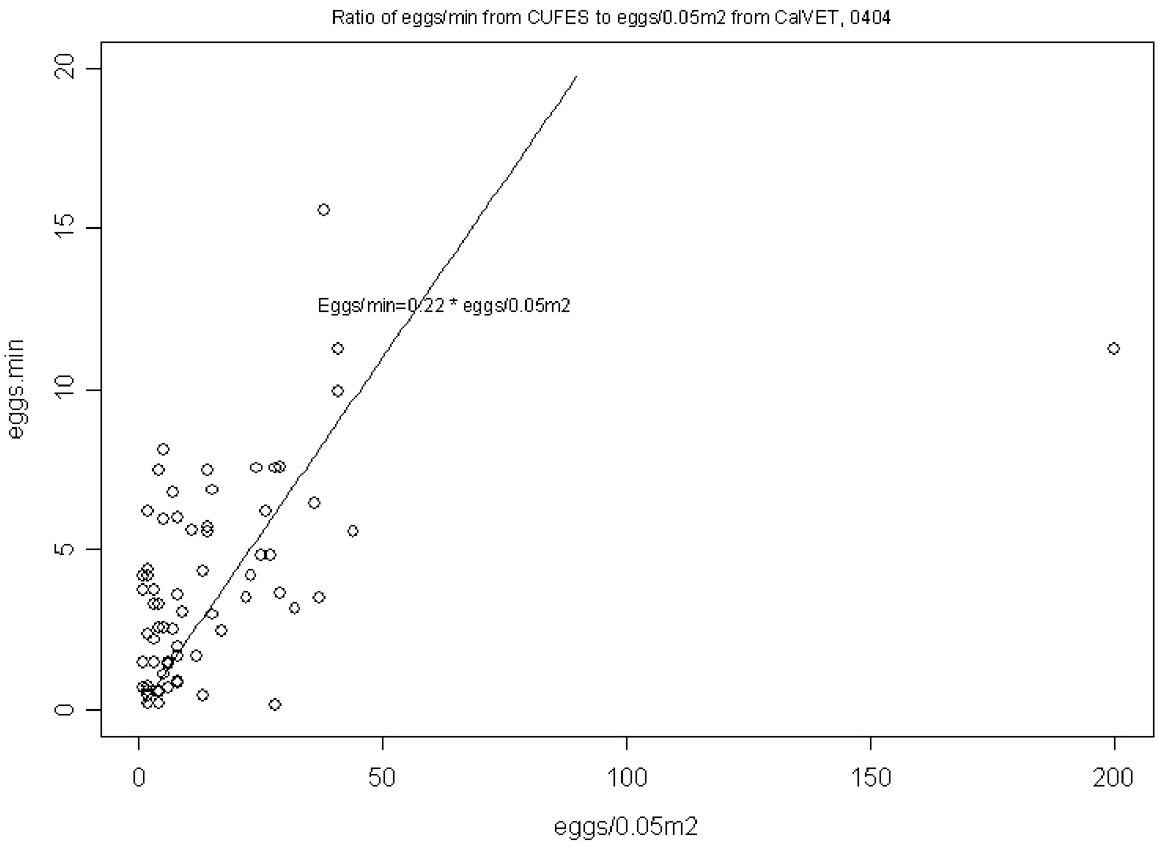

Figure 7. Catch ratio of eggs/min from CUFES to eggs/0.05m^2 from CalVET during March-April 2004.

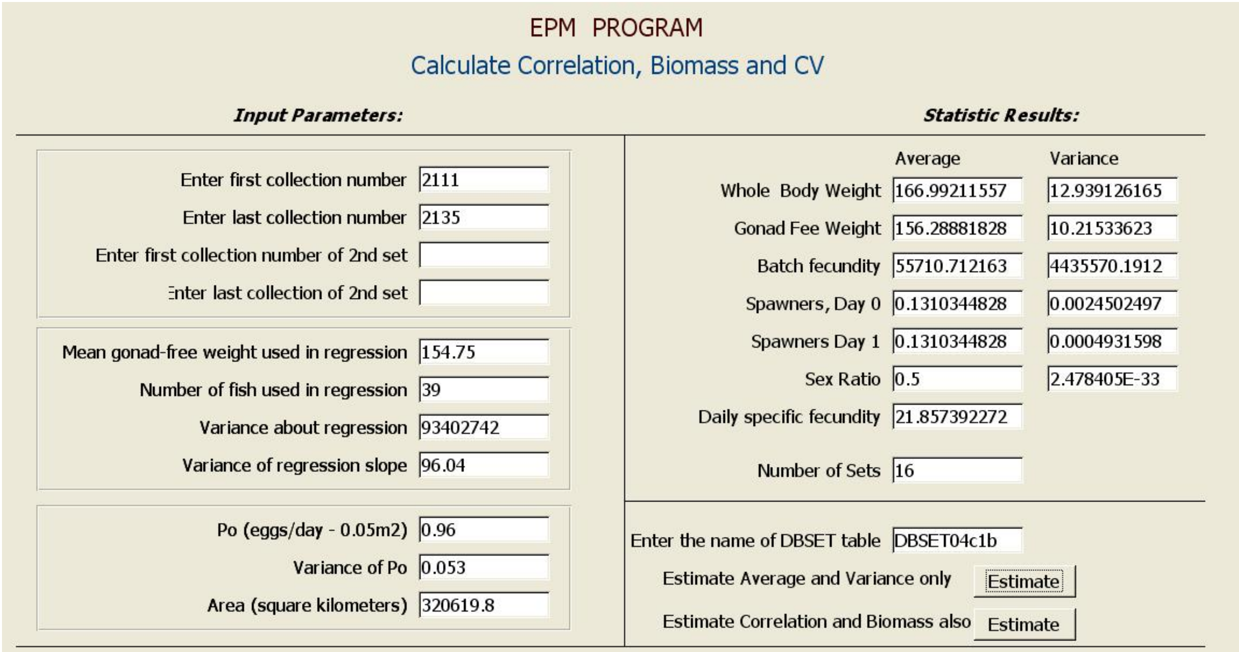

Figure 8. Result after 2004 parameters were entered and 'Estimate Average and Variance only' for adult parameters button was selected in "frmBIOMASS" form using ACCESS program EPM (Appendix II-23 in Chen et al. 2003).

Appendix

Estimate of spawning biomass of Pacific sardine for 1995.

Data of sardine eggs were collected from 42 CalVET net tows (Figure A1) in April CalCOFI survey aboard R/V *New Horizon* from April 6-22 up to CalCOFI station 70 in 1995. For simplicity, this survey area was considered as one region and spawning biomass was computed for this region. Out of 42 stations, 13 stations had caught at least one sardine egg. The density of sardine eggs in each of 11 stages again peaked at stage 6 (Figure A2). For yolk-sac larvae, 8 out 42 CalVET tows are positive (Figure A3). For bongo collections, 17 out of 39 were positive up to station 70. For the whole survey area, 20 out 55 bongo tows had at least one yolk-sac larva (Figure A1).

The embryonic mortality curve (equation 1) was constructed according to the procedure described in the text. A weighted nonlinear regression was used to estimate two parameters in equation (1) where the weights are 1/SD. The SDs of eggs for one day, two day and three day group were 0.53, 2.17 and 2.51 respectively. The SD of yolk-sac larval production from CalVET and Bongo taken from stations $\leq$ 70 was 0.92 and 0.29 respectively. The estimate of P_0 was 0.8/0.05 m^2 (CV=0.5) and the daily mortality rate (z) was -0.4(CV=0.41) (Figure A3). The estimate of P_0 corrected for bias from weighted nonlinear regression was 0.83/0.05 m^2 (Lo 2001).

To obtain the estimate of spawning biomass, we used the estimate of daily specific fecundity computed from the average value of 1986-1994 and estimates of other adult parameters in 1994 (Lo 2003): 23.55 eggs/day/gram of biomass for an area of 113,189 km^2: 79,977 mt (CV=0.6) (Table 5).

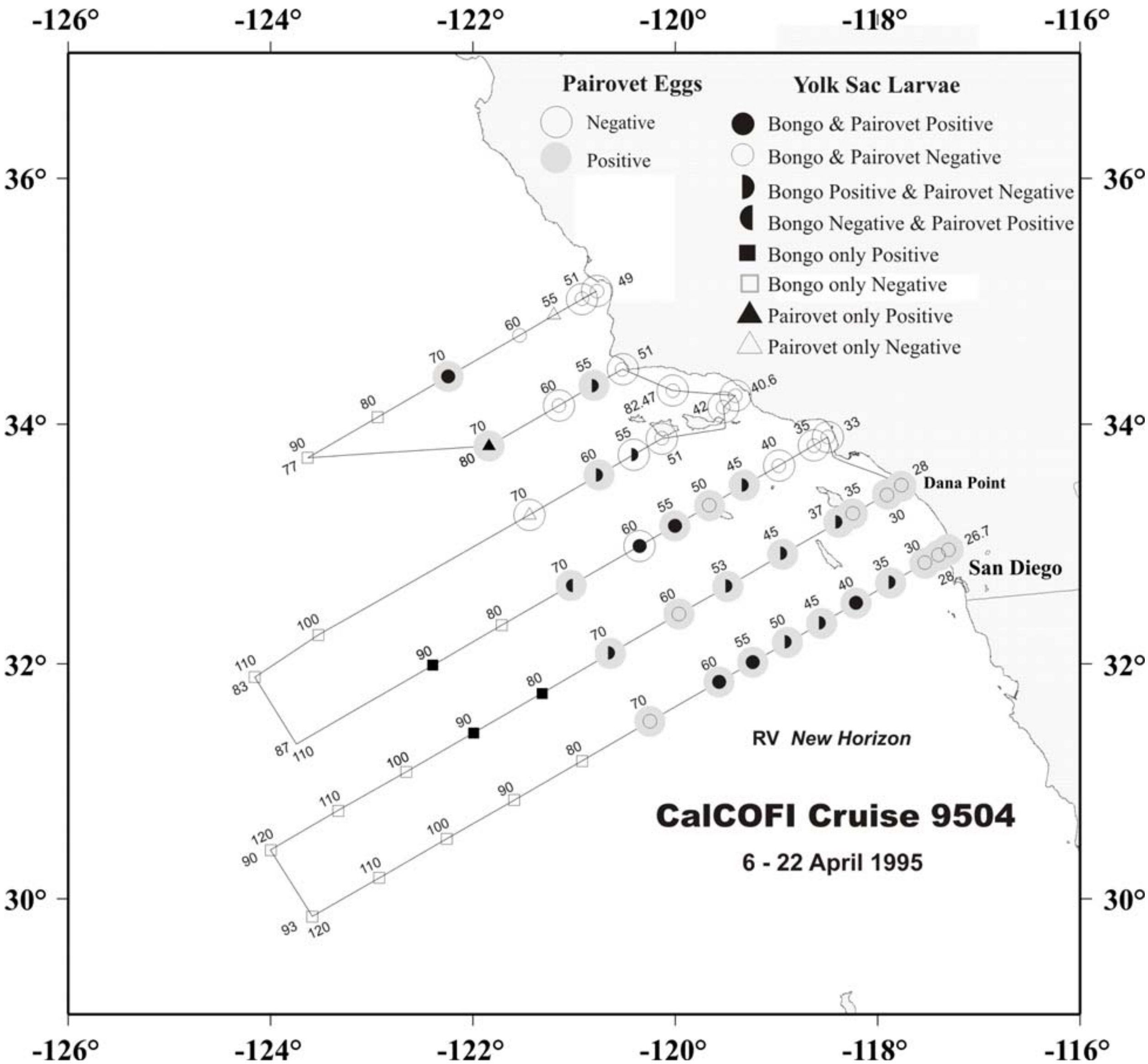

Figure A1. Sardine eggs from CalVET (large circles), yolk-sac larvae from CalVET (or Pairovet; circle and triangle) and from Bongo (circle and square) in April 1995 survey. Solid symbols are positive and open symbols are zero catch.

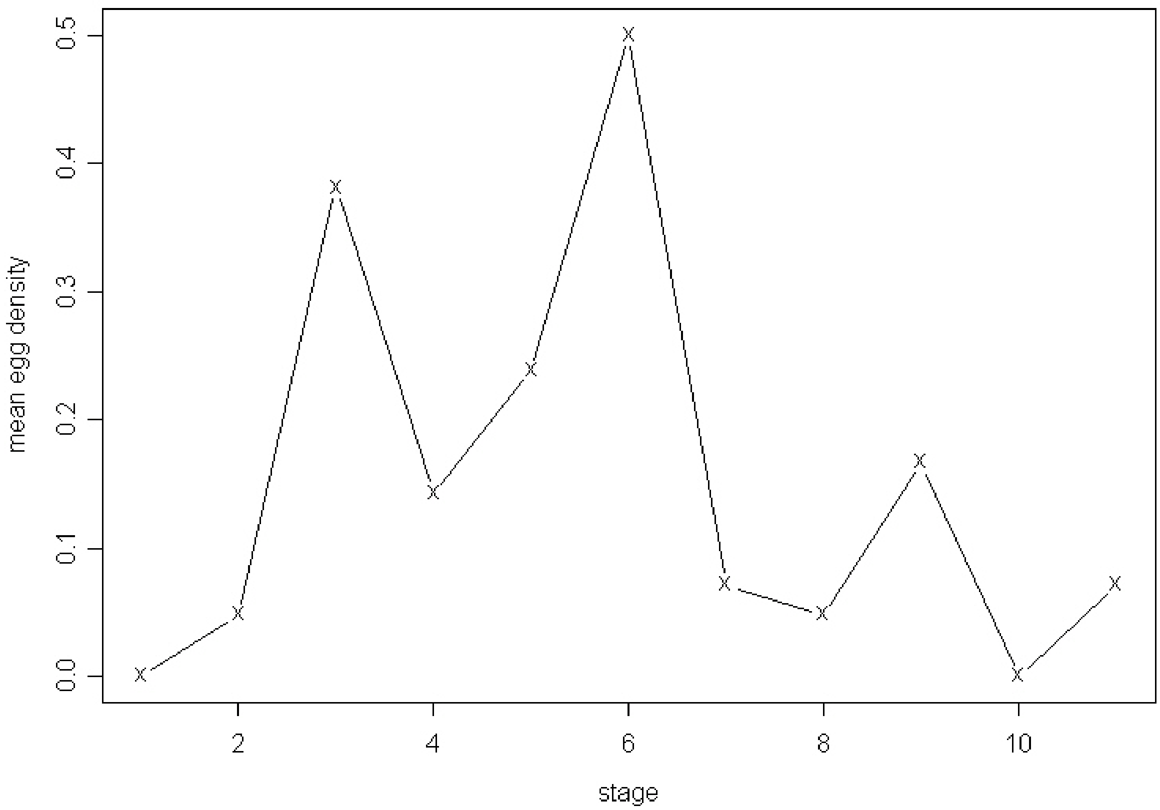

Figure A2. Sardine eggs per $0.05m^2$ for each developmental stage for April 1995.

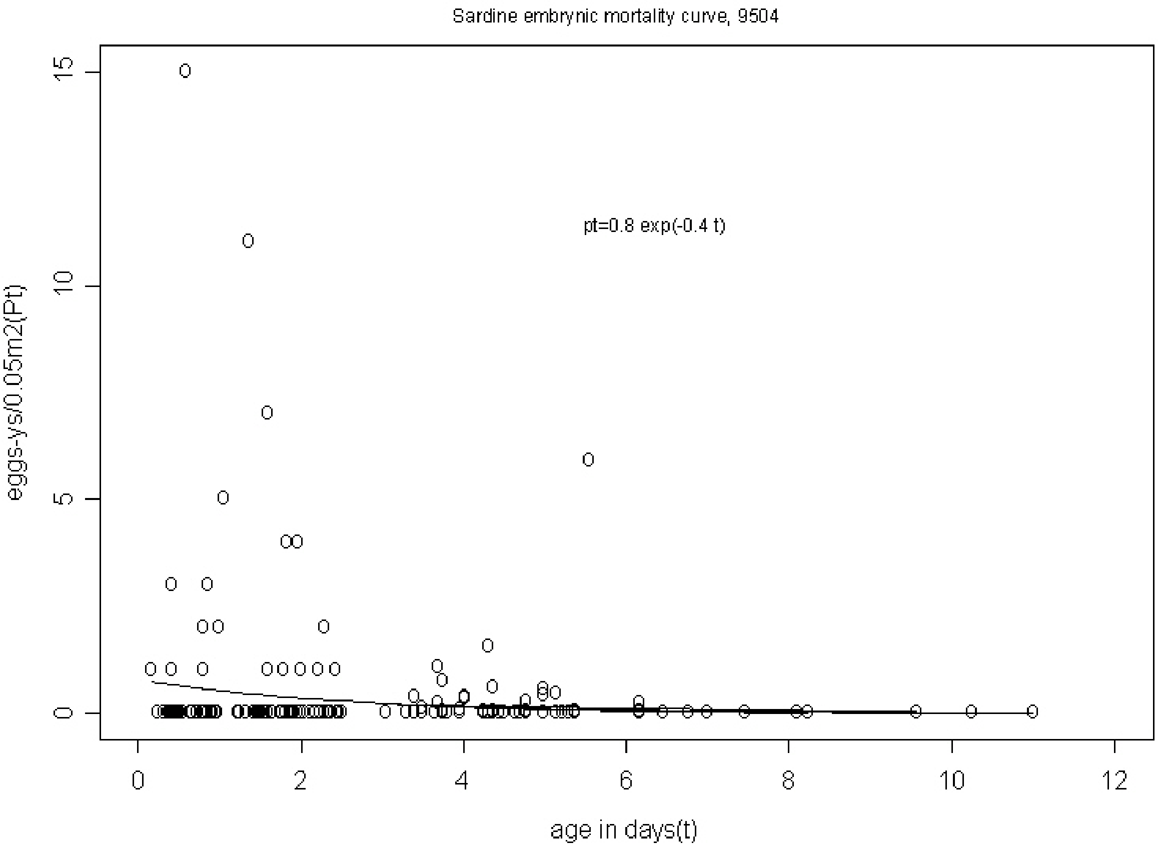

Figure A3. Embryonic mortality curve of Pacific sardine of 1995. Staged egg data were from CalVET and yolk-sac larval data were from CalVET and Bongo. The number, 0.8, is the estimate of daily egg production, before corrected for bias.